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(c) 2004 Mass. Med. Soc.
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*File 467: For information about updating status please see Help News467.

Set	Items	Description

Cost is in DialUnits

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 Set      Items    Description
 S1        1082    AU=LOCHT ?
 S2          40    S1 AND CHOLER?
 S3          19    RD (unique items)
 S4          10    S3 AND (MUTANT? OR MUTATION? OR MUTAGEN? OR SUBSTIT? OR CH-
                     ANGE? OR MODIFIC? OR ALTER? OR INSERT? OR DELET?)
 ?t s4/9/4 3
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File 155:MEDLINE(R) 1966-2004/Feb W5
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Set	Items	Description

?e e29

Ref	Items	Index-term
E1	1	E289D
E2	1	E2896
E3	53	*E29
E4	4	E29A
E5	1	E29C09W09
E6	1	E29D
E7	5	E29H
E8	1	E29K
E9	2	E29L
E10	1	E29Q
E11	1	E290
E12	1	E290A

Enter P or PAGE for more

?s e3-e10

	53	E29
	4	E29A
	1	E29C09W09
	1	E29D
	5	E29H
	1	E29K
	2	E29L
	1	E29Q
S1	67	E3-E10

?s s1 and cholera?

	67	S1
	19539	CHOLERA?
S2	7	S1 AND CHOLERA?

?t s2/9/all

2/9/1
DIALOG(R)File 155:MEDLINE(R)
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12195526 PMID: 12531635
A modified cholera **holotoxin CT-** E29H **enhances systemic and mucosal immune responses to recombinant Norwalk virus-virus like particle vaccine.**
Periwal Sangeeta B; Kourie Kristin R; Ramachandaran Nandini; Blakeney Susan J; DeBruin Sylvia; Zhu Duzhang; Zamb Timothy J; Smith Larry; Udem Steve; Eldridge John H; Shroff Khushroo E; Reilly Patricia A
Department of Viral Vaccine Immunology, Wyeth-Ayerst Research, Pearl River, NY 10965, USA.
Vaccine (Netherlands) Jan 17 2003, 21 (5-6) p376-85, ISSN 0264-410X Journal Code: 8406899
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
In this study, we evaluated the potential of a genetically modified **cholera** toxin, CT- **E29H** as an adjuvant for recombinant Norwalk virus like particle (NV-VLP) vaccine. This detoxified mutant, containing E to H substitution at amino acid 29 of the CT-A1 subunit, was administered with a recombinant Norwalk virus like particle vaccine to Balb/c mice by mucosal routes to monitor the induction of mucosal, humoral and cellular responses. We observed that a low dose of NV-VLP (5 microg) with the adjuvant delivered by the intranasal route (IN) was more effective than the highest dose (200 microg) delivered by oral route at inducing both cellular and

IgA secreting cells were observed in the Peyer's Patches (PP) following delivery of the vaccine with CT- **E29H** as compared to delivery of vaccine by mucosal routes without CT- **E29H** . Furthermore, there was an increase in antigen specific cells producing IL-4 from animals that received the vaccine with the adjuvant. Delivery of the vaccine by the oral route results in antigen specific CD4(+) and CD8(+) T cells in PP and spleen. Addition of CT- **E29H** results in an increase of antigen specific CD4(+) cell population in PP and both CD4(+) and CD8(+) populations in the spleen. These cellular and cytokine responses suggest that combining the vaccine with CT- **E29H** results in a stronger Th2 type response. Collectively, these results indicate that immune responses to NV-VLP vaccine are qualitatively and quantitatively improved when the vaccine is delivered along with CT- **E29H** , and thus merits its further consideration as a mucosal adjuvant.

Tags: Female

Descriptors: Adjuvants, Immunologic--pharmacology--PD; * **Cholera** Toxin --immunology--IM; * **Cholera** Toxin--pharmacology--PD; *Immunity, Mucosal --immunology--IM; *Norovirus--immunology--IM; *Norwalk virus--immunology --IM; *Viral Vaccines--immunology--IM; Adjuvants, Immunologic --administration and dosage--AD; Administration, Intranasal; Animals; Antibody Formation--immunology--IM; Cell Division--drug effects--DE; Enzyme-Linked Immunosorbent Assay; Immunoglobulin A--immunology--IM; Interferon Type II--analysis--AN; Interleukin-4--analysis--AN; Interleukin-4--metabolism--ME; Lymphocytes--drug effects--DE; Lymphoid Tissue--immunology--IM; Mice; Mice, Inbred BALB C; Organ Culture; Vaccines, Synthetic--administration and dosage--AD; Vaccines, Synthetic--immunology --IM; Viral Vaccines--administration and dosage--AD

CAS Registry No.: 0 (Adjuvants, Immunologic); 0 (Immunoglobulin A); 0 (Vaccines, Synthetic); 0 (Viral Vaccines); 207137-56-2 (Interleukin-4) ; 82115-62-6 (Interferon Type II); 9012-63-9 (Cholera Toxin)

Enzyme No.: EC 2.4.2.31 (**cholera** holotoxin, His(29)-)

Record Date Created: 20030117

Record Date Completed: 20030902

2/9/2

DIALOG(R)File 155:MEDLINE(R)

12034593 PMID: 12355362

Immunization with Haemophilus influenzae Hap adhesin protects against nasopharyngeal colonization in experimental mice.

Cutter David; Mason Kathryn W; Howell Alan P; Fink Doran L; Green Bruce A ; St Geme Joseph W

Edward Mallinckrodt Department of Pediatrics, Washington University School of Medicine, 660 S. Euclid Avenue, St. Louis, MO 63110, USA.

Journal of infectious diseases (United States) Oct 15 2002, 186 (8) p1115-21, ISSN 0022-1899 Journal Code: 0413675

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: AIM; INDEX MEDICUS

Nontypeable Haemophilus influenzae is a common cause of respiratory tract disease and initiates infection by colonizing the nasopharynx. The H. influenzae Hap adhesin is an autotransporter protein that was discovered because it promotes intimate interaction with human epithelial cells. Hap contains an extracellular domain called Hap(s) that has adhesive and protease activity and an outer membrane domain called Hap(beta) that serves to present Hap(s) on the surface of the cell. Hap(s) purified from nontypeable H. influenzae strain P860295 was used to immunize BALB/c mice intranasally. Immunization stimulated significant mucosal and serum anti-Hap(s) antibody titers, which were augmented by the addition of mutant **cholera** toxin (CT- **E29H**) as an adjuvant. Immunization was associated with a marked reduction in the density of nasopharyngeal colonization when mice were challenged with a heterologous strain of nontypeable H. influenzae. These results suggest that intranasal immunization with Hap formulated with CT- **E29H** may be a valuable vaccine strategy for the prevention of nontypeable H. influenzae disease.

Descriptors: *Bacterial Outer Membrane Proteins--immunology--IM; *Haemophilus Infections--immunology--IM; *Haemophilus Infections --prevention and control--PC; *Haemophilus influenzae--immunology--IM; *Nasopharynx--immunology--IM; *Nasopharynx--microbiology--MI; Adjuvants, Immunologic--administration and dosage--AD; Administration, Intranasal; Animals; Antibodies, Bacterial--immunology--IM; Bacterial Adhesion --immunology--IM; Bacterial Outer Membrane Proteins--administration and dosage--AD; Bacterial Outer Membrane Proteins--genetics--GE; Blotting, Western; Cell Line; Cloning, Molecular; Enzyme-Linked Immunosorbent Assay; Epithelial Cells--immunology--IM; Immunity, Mucosal--immunology--IM; Immunization; Immunoglobulin A--immunology--IM; Immunoglobulin G --immunology--IM; Mice; Mice, Inbred BALB C

CAS Registry No.: 0 (Adjuvants, Immunologic); 0 (Antibodies, Bacterial); 0 (Bacterial Outer Membrane Proteins); 0 (Hap protein); 0 (Immunoglobulin A); 0 (Immunoglobulin G)

Record Date Created: 20020930

Record Date Completed: 20021108

Date of Electronic Publication: 20020916

2/9/3

DIALOG(R)File 155:MEDLINE(R)

11308883 PMID: 11395467

Biological and biochemical characterization of variant A subunits of cholera **toxin constructed by site-directed mutagenesis.**

Jobling M G; Holmes R K

Department of Microbiology, University of Colorado Health Sciences Center, Denver, Colorado 80220, USA.

Journal of bacteriology (United States) Jul 2001, 183 (13) p4024-32, ISSN 0021-9193 Journal Code: 2985120R

Contract/Grant No.: AI31940; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Cholera toxin (CT) is the prototype for the Vibrio **cholerae** -Escherichia coli family of heat-labile enterotoxins having an AB5 structure. By substituting amino acids in the enzymatic A subunit that are highly conserved in all members of this family, we constructed 23 variants of CT that exhibited decreased or undetectable toxicity and we characterized their biological and biochemical properties. Many variants exhibited previously undescribed temperature-sensitive assembly of holotoxin and/or increased sensitivity to proteolysis, which in all cases correlated with exposure of epitopes of CT-A that are normally hidden in native CT holotoxin. Substitutions within and deletion of the entire active-site-occluding loop demonstrated a prominent role for His-44 and this loop in the structure and activity of CT. Several novel variants with wild-type assembly and stability showed significantly decreased toxicity and enzymatic activity (e.g., variants at positions R11, I16, R25, **E29** , and S68+V72). In most variants the reduction in toxicity was proportional to the decrease in enzymatic activity. For substitutions or insertions at **E29** and Y30 the decrease in toxicity was 10- and 5-fold more than the reduction in enzymatic activity, but for variants with R25G, E110D, or E112D substitutions the decrease in enzymatic activity was 12- to 50-fold more than the reduction in toxicity. These variants may be useful as tools for additional studies on the cell biology of toxin action and/or as attenuated toxins for adjuvant or vaccine use.

Tags: Comparative Study; Support, U.S. Gov't, P.H.S.

Descriptors: **Cholera** Toxin--genetics--GE; * **Cholera** Toxin--toxicity --TO; ADP-Ribosylation Factors--genetics--GE; ADP-Ribosylation Factors --immunology--IM; ADP-Ribosylation Factors--toxicity--TO; Amino Acid Sequence; Bacterial Toxins--genetics--GE; Bacterial Toxins--toxicity--TO; Binding Sites; **Cholera** Toxin--immunology--IM; Conserved Sequence; Enterotoxins--genetics--GE; Enterotoxins--toxicity--TO; Enzyme Stability; Epitopes; Models, Molecular; Mutagenesis, Site-Directed; Protein

CAS Registry No.: 0 (Bacterial Toxins); 0 (Enterotoxins); 0 (Epitopes); 0 (enterotoxin LT); 9012-63-9 (Cholera Toxin)
Enzyme No.: EC 3.6.1.47 (ADP-Ribosylation Factors)
Record Date Created: 20010607
Record Date Completed: 20010712

2/9/4
DIALOG(R)File 155:MEDLINE(R)

11270507 PMID: 11349048
Recombinant PhpA protein, a unique histidine motif-containing protein from Streptococcus pneumoniae, protects mice against intranasal pneumococcal challenge.
Zhang Y; Masi A W; Barniak V; Mountzouros K; Hostetter M K; Green B A
Department of Immunology, Wyeth Lederle Vaccines, West Henrietta, New York 14586-9728, USA. zhangy4@war.wyeth.com
Infection and immunity (United States) Jun 2001, 69 (6) p3827-36, ISSN 0019-9567 Journal Code: 0246127
Contract/Grant No.: AI 24162; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
The multivalent pneumococcal conjugate vaccine is effective against both systemic disease and otitis media caused by serotypes contained in the vaccine. However, serotypes not covered by the current conjugate vaccine may still cause pneumococcal disease. To address these serotypes and the remaining otitis media due to Streptococcus pneumoniae, we have been evaluating antigenically conserved proteins from S. pneumoniae as vaccine candidates. A previous report identified a 20-kDa protein with putative human complement C3-proteolytic activity. By utilizing the publicly released pneumococcal genomic sequences, we found the gene encoding the 20-kDa protein to be part of a putative open reading frame of approximately 2,400 bp. We recombinantly expressed a 79-kDa fragment (rPhpA-79) that contains a repeated HxxHxH motif and evaluated it for vaccine potential. The antibodies elicited by the purified rPhpA-79 protein were cross-reactive to proteins from multiple strains of S. pneumoniae and were against surface-exposed epitopes. Immunization with rPhpA-79 protein adjuvanted with monophosphoryl lipid A (for subcutaneous immunization) or a mutant **cholera** toxin, CT- **E29H** (for intranasal immunization), protected CBA/N mice against death and bacteremia, as well as reduced nasopharyngeal colonization, following intranasal challenge with a heterologous pneumococcal strain. In contrast, immunization with the 20-kDa portion of the PhpA protein did not protect mice. These results suggest that rPhpA-79 is a potential candidate for use as a vaccine against pneumococcal systemic disease and otitis media.
Tags: Human; Male; Support, U.S. Gov't, P.H.S.
Descriptors: *Bacterial Proteins--genetics--GE; *Endopeptidases --immunology--IM; *Otitis Media--prevention and control--PC; *Pneumococcal Infections--prevention and control--PC; *Pneumococcal Vaccines--immunology --IM; *Streptococcal Vaccines; *Streptococcus pneumoniae--immunology--IM; Administration, Intranasal; Animals; Antibodies, Bacterial--blood--BL; Bacterial Proteins--immunology--IM; Endopeptidases--chemistry--CH; Endopeptidases--genetics--GE; Endopeptidases--metabolism--ME; Histidine --chemistry--CH; Immunization; Mice; Mice, Inbred CBA; Molecular Sequence Data; Nasopharynx--microbiology--MI; Otitis Media--microbiology--MI; Pneumococcal Infections--microbiology--MI; Recombinant Proteins--genetics --GE; Recombinant Proteins--immunology--IM; Recombinant Proteins --metabolism--ME; Sequence Analysis, DNA
Molecular Sequence Databank No.: GENBANK/AF340221; GENBANK/AF340222; GENBANK/AF340223
CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Bacterial Proteins); 0 (PhpA protein, Streptococcus pneumoniae); 0 (Pneumococcal Vaccines); 0 (Recombinant Proteins); 0 (Streptococcal Vaccines); 71-00-1 (Histidine)

Record Date Created: 20010511
Record Date Completed: 20010628

2/9/5
DIALOG(R)File 155:MEDLINE(R)

11231068 PMID: 11270595

Protective efficacy of rotavirus 2/6-virus-like particles combined with CT- E29H , a detoxified cholera **toxin adjuvant.**

Siadat-Pajouh M; Cai L
Department of Viral Vaccine Research, Wyeth-Lederle Vaccines, Pearl River, New York, USA.

Viral immunology (United States) 2001, 14 (1) p31-47, ISSN 0882-8245 Journal Code: 8801552
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

Identifying a safe and efficacious mucosal adjuvant is crucial for the development of subunit vaccines against rotavirus and other mucosal pathogens. Moreover, recognition of determinants of protective immunity to rotavirus infection is essential to the design of the means to prevent or control this viral gastrointestinal disease. We have studied the kinetics of systemic and mucosal antibody responses elicited upon mucosal immunization of mice with rotavirus recombinant virus-like particles (rVLPs) alone or combined with a detoxified version of **cholera** toxin, CT- **E29H** . CT- **E29H** has been shown to maintain the adjuvant effect of parental **cholera** holotoxin. Both inbred BALB/c and outbred CD-1 mice were immunized with rotavirus VP2/6-rVLPs (2/6-VLPs) combined with CT- **E29H** , orally or intranasally (i.n.), and the comparative efficacy of different formulations was then determined. Rotavirus-specific serum and fecal IgA, IgM, and IgG antibodies were determined by enzyme-linked immunoadsorbent assay (ELISA) weekly (or every other week) following vaccination. Animals then were challenged with a murine rotavirus strain, EDIM. The degree to which vaccinated animals were protected from the wild-type rotavirus challenge was reflected in the levels of viral antigen shed in stools (percent reduction in antigen shedding, PRAS). BALB/c mice immunized by either route produced rotavirus-specific serum IgA, IgM and IgG, as well as fecal IgA and IgG, but not IgM; however, the intranasal immunization induced stronger systemic IgG and IgM responses than did oral immunization. Similar levels of prechallenge rotavirus-specific fecal and serum IgA were detected in both the orally and the i.n. immunized groups. Two immunizations with 2-6VLPs and CT- **E29H** were sufficient to protect BALB/c mice, regardless of the route of administration. PRAS was 99.6, 98.8, and 98.8% for oral, i.n. and the oral + i.n. groups, respectively; in contrast vaccination with 2/6-VLPs alone was not protective (PRAS = 39%), indicating the critical role of CT- **E29H** in inducing protective levels of immune responses. Two of four outbred CD-1 mice that were immunized orally with 2/6-VLPs-CT- **E29H** showed no humoral responses (PRAS, 65%), but four of four i.n. immunized CD-1 mice displayed humoral responses (PRAS, 97.9%). Serum anti-VP6 and VP2 antibodies were detected in all immunoresponsive mice. The combined results in two strains of mice indicate that CTE29H is an effective mucosal adjuvant capable of inducing protective immune responses and suggest that intranasal administration is the preferred route of immunization.

Tags: Human
Descriptors: Capsid--immunology--IM; * **Cholera** Toxin--immunology--IM; *Rotavirus Infections--prevention and control--PC; *Rotavirus Vaccines --immunology--IM; Adjuvants, Immunologic; Animals; Antibodies, Viral--blood --BL; Capsid--genetics--GE; Capsid Proteins; Disease Models, Animal; Feces --chemistry--CH; Immunization; Immunoglobulin A, Secretory--analysis--AN; Mice; Mice, Inbred BALB C; Recombinant Proteins--immunology--IM; Rotavirus --immunology--IM; Rotavirus Infections--immunology--IM; Rotavirus Vaccines --administration and dosage--AD; Virion--genetics--GE; Virion--immunology --IM

0 (Capsid Proteins); 0 (Immunoglobulin A, Secretory); 0 (Recombinant Proteins); 0 (Rotavirus Vaccines); 0 (VP2 protein, Rotavirus); 0 (VP6 protein, Rotavirus); 9012-63-9 (Cholera Toxin)
Record Date Created: 20010328
Record Date Completed: 20010816

2/9/6
DIALOG(R)File 155:MEDLINE(R)

10671498 PMID: 10781860
Effective mucosal immunization against respiratory syncytial virus using purified F protein and a genetically detoxified cholera **holotoxin, CT-E29H** .
Tebbey P W; Scheuer C A; Peek J A; Zhu D; LaPierre N A; Green B A; Phillips E D; Ibraghimov A R; Eldridge J H; Hancock G E
Department of Immunology Research, Wyeth-Lederle Vaccines, 211 Bailey Road, West Henrietta, NY 14586-9728, USA.
Vaccine (ENGLAND) Jun 1 2000, 18 (24) p2723-34, ISSN 0264-410X Journal Code: 8406899
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
We exploited the powerful adjuvant properties of **cholera** holotoxin (CT) to create a mucosally administered subunit vaccine against respiratory syncytial virus (RSV). A genetically detoxified mutant CT with an E to H substitution at amino acid 29 of the CT-A1 subunit (CT- **E29H**) was compared to wild type CT for toxicity and potential use as an intranasal (IN) adjuvant for the natural fusion (F) protein of RSV. When compared to CT the results demonstrated that: (1) CT- **E29H** binding to GM1 ganglioside was equivalent, (2) ADP-ribosylation of agmatine was 11.7%, and (3) toxicity was attenuated in both Y-1 adrenal (1.2%) and patent mouse gut weight assays. IN vaccination with F protein formulated with CT- **E29H** induced serum anti-CT and anti-F protein antibodies that were comparable to those obtained after vaccination with equivalent doses of CT. Vaccinations containing CT- **E29H** at doses of 0.1 microg were statistically equivalent to 1.0 microg in enhancing responses to F protein. Antigen-specific mucosal IgA and anti-RSV neutralizing antibodies were detected in nasal washes and sera, respectively, of mice that had received F protein and 0.1 or 1.0 microg of CT- **E29H** . Anti-F protein IgA was not detected in the nasal washes from mice IN vaccinated with 0.01 microg CT- **E29H** or IM with F protein adsorbed to AlOH adjuvant. In addition, the formulation of purified F protein and CT- **E29H** (0.1 and 1.0 microg) facilitated protection of both mouse lung and nose from live RSV challenge. Collectively, the data have important implications for vaccine strategies that use genetically detoxified mutant **cholera** holotoxins for the mucosal delivery of highly purified RSV antigens.
Tags: Female
Descriptors: Antigens, Viral--immunology--IM; * **Cholera** Toxin --immunology--IM; *HN Protein; *Respiratory Syncytial Viruses--immunology --IM; *Viral Proteins--immunology--IM; *Viral Vaccines--immunology--IM; Animals; Bronchoalveolar Lavage; Electrophoresis, Polyacrylamide Gel; Enzyme-Linked Immunosorbent Assay; Immunity, Mucosal; Lung--virology--VI; Mice; Mice, Inbred BALB C; Nasal Mucosa--virology--VI
CAS Registry No.: 0 (Antigens, Viral); 0 (HN Protein); 0 (Viral Proteins); 0 (Viral Vaccines); 0 (attachment protein G); 0 (respiratory syncytial virus proteins); 9012-63-9 (Cholera Toxin)
Record Date Created: 20000711
Record Date Completed: 20000711

2/9/7
DIALOG(R)File 155:MEDLINE(R)

Importance of ADP-ribosylation in the morphological changes of PC12 cells induced by cholera **toxin.**

Glineur C; Locht C
Unite d'Oncologie Moleculaire, CNRS URA 1160, Institut Pasteur, Lille, France.

Infection and immunity (UNITED STATES) Oct 1994, 62 (10) p4176-85, ISSN 0019-9567 Journal Code: 0246127
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

Cholera toxin (CTX) is composed of two subunits, subunit A, which possesses ADP-ribosyltransferase activity, and subunit B, which is responsible for receptor binding. It has previously been shown that agents that increase cyclic AMP (cAMP) levels in cells induce differentiation of PC12 cells into neurite-like cells. In this report, we show that as little as 100 pg of CTX per ml induces such changes. CTX was found to ADP-ribosylate at least four membrane proteins of PC12 cells in vitro and in vivo and to increase intracellular cAMP levels. We have developed an inducible ctx gene expression system in Vibrio **cholerae** by using the tac promoter. The culture medium of the CTX-producing bacteria was able to induce the morphological changes and the ADP-ribosylation of the PC12 cell membrane proteins. We have constructed two CTX-cross-reactive mutant proteins (CTX-CRM) by site-directed mutagenesis. The choice of glutamic acid 29 as the target amino acid was based on sequence similarities with other bacterial toxins. CTX-CRM- **E29** delta, in which the Glu-29 of the A subunit was deleted, showed strongly reduced ADP-ribosyltransferase activity and did not induce significant morphological changes of PC12 cells. In contrast, CTX-CRM- **E29D** , in which the Glu-29 was replaced by an aspartic acid, was as active as the wild-type protein. We conclude that the ADP-ribosylation activity of CTX is important for the toxin-induced differentiation of PC12 cells. Pertussis toxin, which had no visible effect on PC12 cell morphology, was also able to ADP-ribosylate a membrane-bound protein(s) in vitro and in vivo. Pertussis toxin alone did not significantly increase cAMP levels in PC12 cells, but it acted synergistically with CTX.

Tags: Support, Non-U.S. Gov't
Descriptors: Adenosine Diphosphate Ribose--metabolism--ME; * **Cholera** Toxin--toxicity--TO; Amino Acid Sequence; Animals; Base Sequence; CHO Cells; **Cholera** Toxin--biosynthesis--BI; **Cholera** Toxin--genetics--GE; Cyclic AMP--analysis--AN; Forskolin--pharmacology--PD; Genetic Vectors; Hamsters; Molecular Sequence Data; PC12 Cells--drug effects--DE; PC12 Cells--metabolism--ME; Rabbits; Rats; Recombinant Proteins--biosynthesis --BI; Recombinant Proteins--toxicity--TO
CAS Registry No.: 0 (Genetic Vectors); 0 (Recombinant Proteins); 20762-30-5 (Adenosine Diphosphate Ribose); 60-92-4 (Cyclic AMP); 66428-89-5 (Forskolin); 9012-63-9 (Cholera Toxin)
Record Date Created: 19941104
Record Date Completed: 19941104

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$1.47 7 Type(s) in Format 9
$1.47 7 Types
$3.08 Estimated cost File155
$0.24 TELNET
$3.32 Estimated cost this search
$4.45 Estimated total session cost 0.942 DialUnits

Status: Signed Off. (2 minutes)

08578962 PMID: 2363691

Photolabelling of mutant **forms of the S1 subunit of pertussis toxin with NAD+.**

Cieplak W; **Locht C** ; Mar V L; Burnette W N; Keith J M

Laboratory of Pathobiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, MT 59840.

Biochemical journal (ENGLAND) Jun 15 1990, 268 (3) p547-51, ISSN 0264-6021 Journal Code: 2984726R

Contract/Grant No.: IAI82679; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

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The S1 subunit of pertussis toxin catalyses the hydrolysis of NAD+ (NAD+ glycohydrolysis) and the NAD(+)-dependent ADP-ribosylation of guanine-nucleotide-binding proteins. Recently, the S1 subunit of pertussis toxin was shown to be photolabelled by using radiolabelled NAD+ and u.v.; the primary labelled residue was Glu-129, thereby implicating this residue in the binding of NAD+. Studies from various laboratories have shown that the N-terminal portion of the S1 subunit, which shows sequence similarity to **cholera** toxin and Escherichia coli heat-labile toxin, is important to the maintenance of both glycohydrolase and transferase activity. In the present study the photolabelling technique was applied to the analysis of a series of recombinant-derived S1 molecules that possessed **deletions** or **substitutions** near the N-terminus of the S1 molecule. The results revealed a positive correlation between the extent of photolabelling with NAD+ and the magnitude of specific NAD+ glycohydrolase activity exhibited by the **mutants** . Enzyme kinetic analyses of the N-terminal **mutants** also identified a **mutant** with substantially reduced activity, a depressed photolabelling efficiency and a markedly increased Km for NAD+. The results support a direct role for the N-terminal region of the S1 subunit in the binding of NAD+, thereby providing a rationale for the effect of **mutations** in this region on enzymic activity.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: *NAD--metabolism--ME; *Pertussis Toxin; *Virulence Factors, Bordetella--metabolism--ME; Macromolecular Systems; **Mutation** ; N-Glycosyl Hydrolases--metabolism--ME; NAD+ Nucleosidase; Recombinant Proteins --genetics--GE; Recombinant Proteins--metabolism--ME; Recombinant Proteins--radiation effects--RE; Structure-Activity Relationship; Ultraviolet Rays; Virulence Factors, Bordetella--genetics--GE; Virulence Factors, Bordetella--radiation effects--RE

CAS Registry No.: 0 (Macromolecular Systems); 0 (Recombinant Proteins) ; 0 (Virulence Factors, Bordetella); 53-84-9 (NAD)

Enzyme No.: EC 2.4.2.31 (Pertussis Toxin); EC 3.2.2.- (N-Glycosyl Hydrolases); EC 3.2.2.5 (NAD+ Nucleosidase)

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